CLAIMS

What is claimed is:

1. A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding a proteolytic tryptase having an active site mutation, and wherein the expression construct drives expression of a mature proteolytic tryptase that lacks enzymatic activity in hosts transformed to contain the expression construct, the lack of enzymatic activity being due to the active site mutation.

2. The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes β-I tryptase.

3. The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes β-II tryptase.

4. The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes a human proteolytic tryptase.

5. The DNA expression construct according to Claim 1, wherein the active site mutation changes the native amino acid to a non-charged amino acid.

6. The DNA expression construct according to Claim 5, wherein the active site mutation changes the native amino acid to an alanine.

7. The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation has a DNA sequence selected from the group consisting of SEQ. ID. NO. 20, SEQ. ID. NO. 22, SEQ. ID. NO. 24, SEQ. ID. NO. 26, SEQ. ID. NO. 36, SEQ. ID. NO. 38, SEQ. ID. NO. 40, and SEQ. ID. NO. 42.

8. The DNA expression construct according to Claim 1, wherein the proteolytic tryptase has an amino acid sequence selected from the group consisting of SEQ. ID. NO. 21, SEQ. ID. NO. 23, SEQ. ID. NO. 25, SEQ. ID. NO. 27, SEQ. ID. NO. 37, SEQ. ID. NO. 39, SEQ. ID. NO. 41, and SEQ. ID. NO. 43.

9. The DNA expression construct according to Claim 1, wherein the secretion signal sequence encodes a KEX2 cleavage site.

10. The DNA expression construct according to Claim 1, wherein the secretion signal sequence includes a 3' terminus encoding amino acid residues Leu-Glu-Lys-Arg.

11. The DNA expression construct according to Claim 1, wherein the promoter is a constitutive promoter.

12. The DNA expression construct according to Claim 1, wherein the promoter is an inducible promoter.

13. A DNA expression construct comprising, in 5' to 3' order: a promoter selected from the group consisting of *AOX1*, *GAP*, *MOX*, *FMD*, *ADH*, *LAC4*, *XPR2*, *LEU2*, *GAM1*, *PGK1*, *GAL7*, *GADPH*, *CYC1*, and *CUP1*, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding proteolytic tryptase having an active site mutation, the DNA sequence operationally linked to a terminator sequence.

14. The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase encodes β-I tryptase.

15. The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase encodes β-II tryptase.

16. The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes a human proteolytic tryptase.

17. The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation has a DNA sequence selected from the group consisting of SEQ. ID. NO. 20, SEQ. ID. NO. 22, SEQ. ID. NO. 24, SEQ. ID. NO. 26, SEQ. ID. NO. 36, SEQ. ID. NO. 38, SEQ. ID. NO. 40, and SEQ. ID. NO. 42.

18. The DNA expression construct according to Claim 13, wherein the proteolytic tryptase has an amino acid sequence selected from the group consisting of SEQ. ID. NO. 21, SEQ. ID. NO. 23, SEQ. ID. NO. 25, SEQ. ID. NO. 27, SEQ. ID. NO. 37, SEQ. ID. NO. 39, SEQ. ID. NO. 41, and SEQ. ID. NO. 43.

19. The DNA expression construct according to Claim 13, wherein the secretion signal sequence encodes a KEX2 cleavage site.

20. A method of producing an active site mutation of proteolytic tryptases comprising transforming a eukaryotic host cell with an expression construct according to Claim 1, wherein the eukaryotic host cell expresses enzymatically-inactive proteolytic tryptase.

21. The method according to Claim 20, wherein a yeast host cell is transformed.

22. The method according to Claim 21, wherein a host cell of the genus Pichia is transformed.

23. The method according to Claim 22, wherein a *Pichia pastoris* host cell is transformed.

24. The method according to Claim 23, wherein a host cell having the characteristics of *Pichia pastoris* ATCC 20864 or *Pichia pastoris* strain KM71 is transformed.

25. The method according to Claim 20, further comprising isolating the enzymatically-inactive proteolytic tryptase produced.

26. The method according to Claim 20, further comprising screening a library with the enzymatically-inactive proteolytic tryptase produced.

27. The method according to Claim 26, wherein a chemical library is screened.

28. The method according to Claim 26, wherein a peptide library is screened.

29. The method according to Claim 20, further comprising testing a substance with the enzymatically-inactive proteolytic tryptase produced.

30. The method according to Claim 29, wherein the testing is an *in vivo* testing.

31. The method according to Claim 29, wherein the testing is an *in vitro* testing.

32. The method according to Claim 29, wherein the testing is an *ex vivo* testing.

33. The method according to Claim 20, further comprising modeling the enzymatically-inactive proteolytic tryptase produced.

34. A genetically-engineered eukaryotic cell which expresses enzymatically-inactive proteolytic tryptase comprising a eukaryotic host cell transformed to contain and express an expression construct according to Claim 1.

35. The genetically engineered eukaryotic cell of Claim 34, wherein the eukaryotic cell is a yeast cell.

36. The genetically-engineered eukaryotic cell of Claim 35, wherein the yeast cell is of the genus Pichia.

37. A genetically-engineered eukaryotic cell which expresses enzymatically-inactive proteolytic tryptase comprising a eukaryotic host cell transformed to contain and express an expression construct according to Claim 13.

38. A method of generating polyclonal or monoclonal anti-human tryptase antibodies comprising generating antibodies to an enzymatically-inactive proteolytic tryptase according to Claim 20.

39. Polyclonal or monoclonal anti-human proteolytic tryptase antibodies produced according to the method of Claim 38.

40. A recombinant, mature proteolytic tryptase having an active site mutation comprising an amino acid sequence encoding the polypeptide selected from the group consisting of SEQ. ID. NO. 29, SEQ. ID. NO. 31, SEQ. ID. NO. 33, SEQ. ID. NO. 35, SEQ. ID. NO. 45, SEQ. ID. NO. 47, SEQ. ID. NO. 49, and SEQ. ID. NO. 51.

41. A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding proteolytic tryptase, wherein the expression construct drives the expression of mature proteolytic tryptase that has enzymatic activity in hosts transformed to contain the expression construct.

42. The DNA expression construct of Claim 41, wherein the DNA sequence encoding the proteolytic tryptase encodes a human proteolytic tryptase.

43. The DNA expression construct of Claim 42, wherein the DNA sequence comprises SEQ. ID. NO. 8, wherein the expression construct drives the expression of mature β-II tryptase that has enzymatic activity in hosts transformed to contain the expression construct.

44. A method of producing enzymatically-active β-II tryptase comprising transforming a eukaryotic host cell with an expression construct according to Claim 43, wherein the host cell expresses enzymatically-active β-II tryptase.

45. The method according to Claim 44, further comprising isolating the enzymatically-inactive proteolytic tryptase produced.

46. The method according to Claim 44, further comprising screening a library with the enzymatically-inactive proteolytic tryptase produced.

47. The method according to Claim 46, wherein a chemical library is screened.

48. The method according to Claim 46, wherein a peptide library is screened.

49. The method according to Claim 44, further comprising testing a substance with the enzymatically-inactive proteolytic tryptase produced.

50. The method according to Claim 49, wherein the testing is an *in vivo* testing.

51. The method according to Claim 49, wherein the testing is an *in vitro* testing.

52. The method according to Claim 49, wherein the testing is an *ex vivo* testing.

53. The method according to Claim 44, further comprising modeling the enzymatically-inactive proteolytic tryptase produced.

54. The method of Claim 44, wherein a yeast host is transformed.

55. The method of Claim 54, wherein a *Pichia* host is transformed.

56. A genetically-engineered eukaryotic cell which expresses enzymatically-active β-II tryptase comprising a eukaryotic host cell transformed to contain and express an expression construct according to Claim 43.

57. The genetically engineered eukaryotic cell of Claim 56, wherein the eukaryotic cell is a yeast cell.

58. The genetically-engineered eukaryotic cell of Claim 57, wherein the yeast cell is a *Pichia* cell.

59. An enzymatically-active, glycosylated, recombinant human β-II tryptase having tryptase activity produced according to the method recited in Claim 44.

60. A recombinant β-II tryptase comprising an amino acid sequence comprising SEQ. ID. NO. 9.

61. A recombinant, mature β-II tryptase comprising an amino acid sequence comprising SEQ. ID. NO. 11.